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(54) Title: AUTOMATED HISTO-CYTOCHEMISTRY APPARATUS AND ENCAPSULATION SYSTEM FOR PROCESSING BIOLOGICAL MATERIALS

(57) Abstract

There is disclosed an automated apparatus and process for immunocytochemical staining of biological materials using ligand pairing. The apparatus includes an array of sample cells interconnected with reagent supply and drainage lines. Each cell encloses a fluid chamber into which a sample substrate is inserted. The face of the substrate onto which a biological sample is mounted faces into a fluid well. The cell includes a cell head having a chamber to receive therein a solid support matrix releasably containing a ligand to be contacted with the sample. The chamber in the cell head is in flow communication with the fluid well so that when the ligand is released from the matrix it flows into the fluid well thereby contacting the mounted biological sample. The ligand support matrix may be gelatin or wax. The cell body and cell head are differentially heated or cooled so that the mounted sample may be held at the preferred temperature for the reaction of interest and the cell head may be heated to a temperature sufficient to cause thermal degradation of the matrix to effect release of the ligand. Control electronics is provided for sensing and controlling the temperature of the various cell parts and for programmable control of reagent supply to the cells.

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AUTOMATED HISTO-CYTOCHEMISTRY APPARATUS AND ENCAPSULATION SYSTEM FOR PROCESSING BIOLOGICAL MATERIALS

FIELD OF THE INVENTION

The present invention relates to a method and apparatus for processing biological materials utilizing ligand pairing and relates more particularly to the field of microscopical analysis where one member of a ligand pair is to be detected in a biological sample mounted on a support substrate.

BACKGROUND OF THE INVENTION

To examine the structure of biological samples such as tissues (histology) or cells (cytology), microscopical preparations are made by mounting the sample on a substrate such as a microscope slide. These preparations are routinely stained with dyes to facilitate microscopical examination. To further aid in the identification of the samples, specialized procedures under the general headings of histochemistry (tissue slices) and cytochemistry (biological cell smears) are applied to these preparations. One class of procedures for processing biological materials involves ligand-pair formation wherein a first member of the ligand pair may be present in the biological sample and the other member of the pair binds to the first member when contacted with the sample. Examples of such biologically based ligand-pairs include antibody/antigen couples, lectins/sugars, hormone/receptor systems, enzyme/substrates, DNA/DNA and DNA/RNA couples.

Processing of biological materials involving the antibody/antigen couple forms the basis of immunohisto- and immunocytochemistry. Until recently the staining of mounted samples using these reactions has been performed manually. Present machines currently known for immuno-staining of samples dispense the antibody containing solutions in liquid form into the fluid well containing the supported sample. These machines require considerable operator attention which entails high labour costs and are prone to suffer from operator error at the stages of dilution, pipetting and loading of

reagents. Furthermore, in many circumstances it may be desirable to detect different antigens on an ad hoc basis but the primary antibodies are expensive and prone to deterioration. In addition, the difficulties of working with a large number of small volumes used for a multitude of different tests has acted as a bar to the development of an optimally automated immunocytochemistry staining system.

Accordingly, it is desirable to provide a process and apparatus for automated processing of biological materials involving ligand pair formation which avoids the need to prepare antisera immediately prior to use and which does not require accurate positioning and alignment of the sample substrates.

SUMMARY OF THE INVENTION

The present invention provides an apparatus for processing biological materials using ligand pairing, the biological material being mounted on a surface of a substrate. The apparatus comprises a housing provided with at least one cell comprising a first cell portion defining a first chamber having opposed chamber walls. The first chamber is dimensioned to receive the substrate between the opposed chamber walls with the surface of the substrate being adjacent to and spaced from one of the opposed chamber walls a sufficient distance to prevent capillary action from retaining a liquid therebetween. A second cell portion is provided which defines a second chamber adapted to receive a ligand. The second chamber is in fluid flow communication with the first chamber. The apparatus includes reagent supply means for supplying reagent solutions to the first chamber and drainage means for draining said reagent solutions from said first chamber.

In another aspect of the invention there is provided a process for releasing a ligand onto a biological sample mounted on a substrate. The process comprises the steps of containing the ligand in a releasable containment means. The releasable containment means is held in a chamber in flow communication with the biological sample. The releasable containment means is disintegratable if heated to within a predetermined temperature range thereby to release the ligand. The process includes heating the releasable

containment means to within the predetermined temperature range and flowing the ligand onto the biological sample.

In another aspect of the invention there is provided a method of storing a ligand in a releasable containment means comprising mixing a predetermined amount of the ligand with a material which can be formed into a support matrix to encapsulate said ligand, said support matrix being responsive to release means to release the ligand therefrom. In this aspect of the invention the material from which the releasable containment means is made may be wax or gelatin and the ligand released therefrom using heat. Alternatively, the material could be starch or sugars and the ligands released by exposing the support matrix to an aqueous solution.

BRIEF DESCRIPTION OF THE DRAWINGS

The process and apparatus for processing biological materials forming the present invention will now be described, by way of example only, reference being had to the drawings, in which:

Figure 1 is a perspective view of an embodiment of an apparatus for processing biological materials forming part of the subject invention;

Figure 2 is a view along the line 2-2 of Figure 1;

Figure 3 is a clamshell exploded perspective view of the two cells shown in section in Figure 2;

Figure 4 is a schematic representation of an embodiment of a reagent flow system forming part of the apparatus;

Figure 5 is a front view of an embodiment of a containing means which may be used with the apparatus forming the subject invention;

Figure 6 is a perspective view of part of an alternative embodiment of a cell constructed in accordance with the present invention; and

Figure 7 is a cross-sectional side view of the partial cell in Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

Referring first to Figure 1, an automated apparatus 10 for performing the task of processing of biological materials includes a housing or sample compartment 12 comprising a plurality of sample cells 14 arranged in a rectangular array. A storage compartment 16 is located on one side of compartment 12 for housing fluid pumps, valves and reagents and a compartment 18 is located on the other side of compartment 12 for housing electronic control circuitry. An instrument panel comprising a microcomputer keypad 22 and a power switch 24 are shown as part of the control circuitry.

Details of sample cells 14 shown in Figure 1 are more clearly illustrated in Figures 2 and 3. Referring specifically to Figure 3, cells 14 are arranged in rows 30 with each row comprising a plurality of cell blocks 32 attached together. Cell block 32 includes a cell body 34 having on one side thereof a face 36 into which a rectangular shaped recess or well 38 is formed defined by wall 33. Holes 37 and 39 are located along the bottom edge of well 38 and are in flow communication with tubes 41 and 43 respectively which are attached to the bottom of cell body 34 for supplying and draining reagents such as wash buffers to and from the cell.

In another embodiment of the cell, only one hole may be provided (not shown) for both supplying and draining reagents from the chamber. In this arrangement the hole is connected to a tube having a T-junction and valve for providing a reagent inlet and outlet.

The other side of cell body 34 includes a wall 40 having a flange 42 projecting outwardly along three edges thereof. Flange 42 and cell body 34 are dimensioned so that the outer edge 44 of the flange abuts the edges of face 36 of the adjacent cell body 34 when assembled. Cell body 34 includes a liquid overflow comprising a channel 46 extending through the horizontal section of flange 42 and a tube 48 attached to the bottom edge of the flange to drain away liquid.

Cell block 32 is provided with a head portion 50 shown attached to cell body 34 by screws 52. Alternative fastening means such as glues or epoxy may also be used. Cell body 34 and cell head 50 may be fabricated of

a metal such as coated aluminum or stainless steel.

Interposed between cell body 34 and head portion 50 is a thermal insulator 54 for thermally insulating the head from the cell body. Insulator 54 may be fabricated of a plastic such as nylon or TEFLON®. Channels 60 extend through cell body 34 and a channel 62 extends through cell head 50. Channels 60 and 62 are provided for passing fluid through the cell components for heating or cooling. Referring to Figure 3, tubes 68 and 70 are secured to body 34 and head 50 respectively to provide fluid inlets and outlets. Cell body 34 and head 50 may be heated or cooled to the same temperature using a common source of heating fluid or alternatively they may be selectively heated or cooled using separate sources of fluid because of insulator 54 disposed therebetween.

One side of cell head 50 is provided with a surface 80 inclined at about 45 degrees to the horizontal when the cell is assembled. A leaf spring 82 is secured to the other side of cell head 50 coplanar with wall 40 of cell body 34. Spring 82 is bowed outwardly from, and extends downwardly adjacent to, side 40 of cell body 34. The side of head 50 to which spring 82 is attached is provided with flanges 84 projecting outwardly from the side edges, flanges 84 being collinear with the vertical portions of flanges 42 on cell body 34 when the cell head is attached to the body to also sealingly engage face 36. Spring 82 acts to bias substrate 116 against face 36 but other alternative biasing means may be used including a piston arranged to bear against the back surface of the substrate slide.

A front plate 86 is bolted to the adjacent cell block 32 at the front of each row 30. Plate 86 is provided with a planar front face 90 and back face 92 to which spring 82 is attached.

A row of cells is assembled by aligning front plate 86 and a plurality of cell blocks 32 in a row and bolting them together with elongate bolts 110 extending through holes 112 in the plate and similar holes in the cell bodies 34. Alternative means of attaching the cell blocks together may be employed in addition to bolts.

Referring specifically to Figure 2, each cell 14 is formed when

adjacent cell bodies 34 are attached together thereby defining a fluid chamber 114 formed between adjacent cell blocks 32. Chamber 114, which includes well 38, is defined by opposed walls 33 and 40 and is dimensioned to receive substrate 116 with the surface to which the biological material is mounted facing into well 38. Spring 82 acts to bias substrate 116 against cell body 34 with the edges of the substrate overlapping the sides and bottom edge of face 36 on either side of well 38 by about 2 mm to form a seal between the peripheral edge of substrate 116 and face 36.

The internal dimensions of fluid chamber 114 are chosen to ensure that a large enough gap exists between the opposed faces of substrate 116 and the adjacent chamber walls to prevent capillary action. For example, well 38 is of sufficient depth to prevent capillary action from retaining the fluids therein. The minimum allowable dimensions will depend on the material of construction of cell body 34 and on the material wetting properties.

A chamber or receptacle 160 is formed when cell 14 is assembled. Chamber 160 is in flow communication with well 38 when the substrate 116 is in chamber 114. Chamber 160 is dimensioned to receive a support matrix 162 containing a ligand, to be more fully discussed below. Cell head 50 may be constructed with chamber 160 having numerous shapes to act as a receptacle for support matrix 162 as long as there is an unobstructed flow path connecting chamber 160 with well 38.

Referring to Figure 4, a schematic drawing showing one embodiment of a reagent circulation system is shown generally at 130 and comprises a plurality of reagent storage containers 132 stored in reagent storage compartment 16 (Figure 1). Tubes 134 convey the reagents to the input of a reagent selection valve 136 located within the staining module 12, the output of which is fed to a reagent distribution valve 138. Connectors 135 may be standard snap-fit connectors for conveniently connecting and disconnecting reagent containers 132. A plurality of tubes 140 connect valve 138 with chamber inlet control valves 142, one connected to each tube 41 for supplying reagents to each well 38. Drain tubes 43 are connected to lines 150 which feeds into an outlet valve 152, the output of which is fed to a reagent waste

container 154. Other fluid delivery systems will be readily apparent to those skilled in the art. For example, reagent selection valve 136 and reagent distribution valve 138 may be combined into one valve. Alternatively, the function of reagent distribution valve 138 and chamber control valve 142 may be combined into one valve associated with each chamber.

Cell body 34 and head portion 50 may be differentially heated or cooled by water circulated through the body and head by separate pumps and heaters. The pumps and heaters are controlled using standard controllers under microprocessor control. The controllers, pumps and microprocessor may be located in compartments 16 or 18 (Figure 1) or alternatively may be located external to apparatus 10.

Those skilled in the art will appreciate that numerous other embodiments of apparatus 10 may be made. For example, instead of differentially heating with water, small resistance electrical heaters may be embedded into cell body 34 and cell head 50, the heaters being connected to a dual channel electrical power supply. Standard thermocouples may be attached to the two cell components forming part of the control system.

Substrate 116 may be a standard microscope slide, however, other substrates may be used as well. For example, chamber 114 may be designed to receive substrates comprising an electron microscope grid on which a sample is mounted. Other types of substrates will be readily apparent to those skilled in the art.

Referring to Figure 5, an alternative containment or confining means 200 comprises a plastic container sized to fit into receptacle 160 as illustrated generally at 200 in Figure 5. Container 200 comprises a plurality of separate compartments 202 each capable of containing a different solution. At least one of the plurality of separated compartments 202 would contain a solution of a single member of a ligand pair 209, while other compartments may contain solution of an enhancer 211 (i.e. an enzyme solution which enhances results by removing interfering substances) or a solution of one of the several components 213, 215 of the detection system used to detect the ligand previously released and now bound to its complementary partner in the

biological material on the substrate. The open ends 204 of each compartment 202 may be sealed with gelatin or wax sheets 208, 210. 212 and 214 which melt at the same or different temperatures depending on the application for which the ligand pair members are being used.

Referring now to Figures 6 and 7, an alternative cell body 302 has a cell head 304 integrally formed therewith as a single unitary piece. Cell body 302 and head 304 are fabricated of a low thermally conductive material such as TEFLON® or nylon. Cell body 302 includes a well 312 defined by wall 306. Cell head portion 304 includes an inclined surface 314 which acts as a receptacle into which a releasable containment means is inserted in use. Head portion 304 is heated by a heater element 316 embedded below inclined surface 314. Electrical conductors 308 provide power to heater 316. Cell body 302 is heated by a heater element 318 embedded in the body below wall 306. Conductors 310 provide power to heater 318. Cell body 302 and cell head 304 may be selectively heated in this way because cell block 302 is made of a low thermally conductive material.

The fully automated system disclosed herein is advantageous in that it allows the concurrent demonstration of many different antigens when processing the biological samples with antibody/antigen systems. Figure 1 illustrates an embodiment of the apparatus comprising 50 cells 14 but machines may be constructed with more or less cells depending on the anticipated load for the particular application. Apparatus 10 may house adequate stocks of the appropriate solvents, normal sera, secondary and tertiary immuno-labelling reagents and colour developing solutions. In one embodiment the apparatus may comprise a refrigeration unit for the pre-diluted primary and accessory antibodies in order to allow storing of reagents.

The set-up procedure comprises the three steps of: 1) placement of the substrates having the biological samples mounted thereon into cells 14; 2) inserting into chamber 160 a releasable containment means 162 containing at least one member of a ligand pair to be contacted with the biological sample; and 3) initiating the start sequence. The apparatus may be adapted to perform the steps of: blocking endogenous enzyme activity (for peroxidase labelling

systems); proteolysis; blocking non-specific protein binding; multiple antibody incubations with intervening washes; colour development; and possibly counterstaining. The substrates are then manually removed from the machine for coverslipping and microscopical examination. Biological samples in the form of paraffin sections may be manually dewaxed prior to insertion into chamber 114, or alternatively the apparatus may be adapted to perform the steps of dewaxing and hydration. Specifically, with appropriate tubing, electrical insulation and locating the apparatus in a properly ventilated area, the steps of automated dewaxing and hydration of paraffin sections could be performed using known dewaxing and hydration agents such as xylene and alcohol solutions.

In operation, substrate 116, which supports a biological sample on one surface thereof, is inserted into chamber 114 with the sample facing well 38. A releasable containment means 162 (containing a ligand the other member of the pair) is placed in chamber 160. When used with the apparatus having heated components, containment means 162 is preferably a gelatin or wax solid matrix to be described in more detail below. Alternatively, containment means 162 may comprise a hollow capsule made of wax or gelatin. The gelatin and wax matrices are temperature sensitive so that at a certain predetermined temperature the capsule thermally degrades to release its contents. Cell body 34 is maintained at the optimum temperature for rapid and specific ligand pairing procedures. Once the support matrix/capsule 162 has been placed in cell head 50, the temperature of the head may be raised at some point during the procedure, so that the solid support matrix or capsule breaks down thereby releasing the reagent contained therein.

The releasable containment means may be made of a material which can be broken down or decomposed by any one of several physical or chemical processes such as by puncture, electrical, pressure, vacuum or exposure to EM radiation or solvents.

For example, when the releasable containment means is sucrose or starch (amylose or amylopectin) containing antibodies, decomposition may be achieved by exposure to aqueous or aqueous buffered solutions so that

head portion 50 need not be heated. When selecting a releasable containment system for a given ligand, it is important that the ligand and encapsulation material do not irreversibly bind.

Alternatively, chemical processes such as degradation or pH change may be utilized as a release means to break down the matrix, this being accomplished by filling fluid well 38 and chamber 160 with a suitable reagent. When the releasible containment means is a membrane or solid matrix made of glyceryl mono-oleate (MYVEROL 18-99®) then decomposition may be effected by exposure to solutions of pH > 6.8. Release of DNA probes linked to a solid matrix through disulphide bonds may be achieved by dithiothreitol cleavage. Release of DNA probes bound to a solid matrix may also be effected by enzyme release through nuclease-specific auxiliary sequences.

One criterion for selecting the chemical release means is that the other components of the biological system are not adversely affected or interfered with by the chemical. The result of breaking down the matrix is to release the preservative medium containing the solution of antibodies which then flow through the fluid flow path through the cell into contact with the biological sample mounted on the substrate.

When the apparatus is used for immunocytochemical staining applications, the reagent encapsulated within the capsule comprises stabilized antibodies and is more fully discussed herebelow. However, apparatus 10 may be used for other applications involving the pairing of initially separate ligand pairs. Non-limiting examples of such ligand pairs includes lectins/sugars, hormone/receptor systems, enzyme/substrates, DNA/DNA and DNA/RNA couples to mention a few. The member of the ligand pair to be detected in a sample is mounted on substrate 116 within chamber 114 with the sample facing well 38 and the complementary member of the ligand pair is contained within a support matrix/capsule 162 mounted in chamber 160.

Antibody Stabilization And Support

The highly specific nature of antibodies (or immunoglobulins) for recognizing different antigens forms the basis for immunocytochemistry

(immunohistochemistry). In this technique the antigen of interest to be identified in biological samples is purified and an animal is immunized with the antigen thereby eliciting the synthesis of the specific antibodies. These antibodies may then be isolated and applied in-vitro to the sample. The antibodies bind to a site on the recognizable antigen in the sample and the resulting complexes may be detected by light microscopy using labels such as fluorochromes, enzymes such as horseradish peroxidase and alkaline phosphate which in turn may be detected using histochemical reactions or colloidal metal particles which are detected using electron microscopy.

The antibodies are stabilized by being dissolved in a suitable stabilizing medium which in turn is releasably contained within a solid support matrix or capsule. The matrix may be a solid gelatin or wax block or a hollow capsule made of gelatin, wax or plastic which encapsulates the stabilizing medium containing the primary antibodies. The stabilizing medium may comprise a saline solution with a buffering system such as Trisma based buffers, phosphate based buffers, citrate based buffers and the like. The stabilizing medium may also comprise detergents such as TRITON X-100® (octyl phenoxy polyethoxy ethanol), NP40® (octyl phenol ethylene oxide condensate), TWEEN 20® (polyoxyethylene sorbitan monolaurate) and TWEEN 80® (polyoxyethylene sorbitan mono-oleate as well as biological compounds such as proteins, glycolipids, glycoproteins and antimicrobial agents such as thimerosal, sodium azide, sodium metabisulphite and the like. Gelatin is the preferred confining medium for the above referenced solutions while wax is preferred for simple aqueous antibody solutions. Several examples of encapsulation systems are now described.

Example I

In this application, a solution of antibodies in suitable stabilizing medium is incorporated into a gelatin matrix with appropriate physical properties and sensitive to melting by heat at temperatures in for example the 35-45°C range. This matrix has been tested for compatibility with the antibodies, and is composed of a 6% gelatin (300 bloom) in antibody preserving solution. The

antibody in gelatin matrix is formed into a convenient form, and solidified by reducing the temperature. The gelatin matrix may then be handled, shipped, stored, etc. To release the antibodies, the gelatin matrix is destroyed by heating to about 40°C. The matrix melts to form a gelatin/buffer solution which may flow into empty well 38 or mix with a reagent solvent or buffer previously admitted into the well through inlet tube 41 from the reagent supply. Alternatively, the reagent buffer in well 38 may be displaced into chamber 160 by the gelatin/buffer solution or it may be pumped out.

Example II

In this application, the antibodies and subsequent reagents are incorporated into multiple gelatin or wax matrices which melt at temperatures in the 35-50°C range. They are formed into a convenient physical shape, and the reagents are released as in Example I by heating and melting the matrix. In this case, the different matrices are melted at different temperatures, reagent 1 is in a matrix melted at about 40°C, reagent 2 is in a matrix melted at about 45°C, and reagent 3 is in a matrix melted at about 50°C .

Example III

This example is similar to Examples I & II in that the different reagents are released by melting matrices which are sensitive to different temperature ranges. However, the reagents are in a liquid or semi-solid solution which is held in a capsule by the matrix barrier. Melting the matrix destroys the barrier, and the reagent is released into the buffer solution and reacts with the sample.

Those skilled in the art will appreciate that the method of supporting one member of a ligand pair in a support matrix forming part of the present invention is not limited to use with the automated apparatus also forming part of the present invention. For example, the support matrix containing one member of the ligand pair may be placed on a substrate having a sample mounted thereon. The release means, whether heat, solvent etc. is then applied to the support matrix to release the ligand member therefrom to

contact it with the sample.

While the process and apparatus for the automated processing of biological materials forming the present invention has been described and illustrated with respect to the various embodiments, it will be appreciated that numerous variations of these embodiments may be made without departing from the scope of the invention as described herein.

THEREFORE WHAT IS CLAIMED IS:

1. An apparatus for processing biological materials using ligand pairing, the biological material being mounted on a surface of a substrate, comprising:

a) a housing provided with at least one cell, said at least one cell comprising a first cell portion defining a first chamber having opposed chamber walls, the first chamber being dimensioned to receive the substrate between said opposed chamber walls with said surface of the substrate being adjacent to and spaced from one of said opposed chamber walls a sufficient distance to prevent capillary action from retaining a liquid therebetween, a second cell portion defining a second chamber adapted to receive a ligand, the second chamber being in fluid flow communication with said first chamber; and

b) a reagent supply means for supplying reagent solutions to said first chamber and drainage means for draining said reagent solutions from said first chamber.

2. The apparatus according to claim 1 wherein said first cell portion and said second cell portion are separated by a thermal insulator.

3. The apparatus according to claim 2 including means for selectively heating or cooling at least one of said first and second cell portions.

4. The apparatus according to claim 3 wherein said means for heating at least one of said first and second cell portions comprises electrical heating elements operably coupled to said first and second cell portions.

5. The apparatus according to claim 3 including means for sensing the temperature of said first and second cell portions.

6. The apparatus according to claim 5 including water flow passageways extending through said first and second cell portions, wherein said means for

selectively heating said first cell portion and said second cell portion comprises passing water at predetermined temperatures through said water flow passageways in said first and second cell portions.

7. The apparatus according to claim 6 including fluid level sensor means operably coupled to said cells for detecting the level of a fluid in said first chamber.

8. The apparatus according to claim 1 wherein said at least one cell is fabricated of a thermal insulating material.

9. The apparatus according to claim 8 including means for heating or cooling at least one of said first and second cell portions.

10. The apparatus according to claim 9 wherein said means for heating at least one of said first and second cell portions comprises electrical heating elements operably coupled to said first and second cell portions.

11. The apparatus according to claim 10 including means for sensing the temperature of said first and second cell portions.

12. The apparatus according to claim 9 including water flow passageways extending through said first and second cell portions, wherein said means for selectively heating said first cell portion and said second cell portion comprises passing water at predetermined temperatures through said water flow passageways in said first and second cell portions.

13. The apparatus according to claim 8 including fluid level sensor means operably coupled to said at least one cell for detecting the level of a fluid in said first chamber.

14. A process for releasing a ligand onto a biological sample mounted on a

substrate, the process comprising the steps of:

a) containing the ligand in a releasable containment means, the releasable containment means being held in a chamber in flow communication with the biological sample, the releasable containment means being disintegratable if heated to within a predetermined temperature range thereby to release the ligand; and

b) heating the releasable containment means to within said predetermined temperature range and flowing the ligand onto the biological sample.

15. The process according to claim 14 wherein a housing holds the substrate and positions the releasable containment means above the biological sample, the housing having a heating means for heating the releasable containment means to within said predetermined temperature range, said housing having a channel for allowing the released ligand to flow therethrough, the channel conducting the ligand to the biological sample.

16. The process according to claim 15 wherein said releasable containment means is wax.

17. The process according to claim 15 wherein said releasable containment means is gelatin.

18. A method of storing a ligand in a releasable containment means comprising;

mixing a predetermined amount of said ligand with a material which can be formed into a support matrix to encapsulate said ligand, said support matrix being responsive to release means to release said ligand therefrom.

19. The method according to claim 18 wherein said support matrix is a solid support matrix.

20. The method according to claim 18 wherein said support matrix is a membrane encapsulating said ligand.

21. The method according to claim 18 further comprising mixing said ligand with a stabilizing medium prior to encapsulating said ligand.

22. The method according to claim 18 wherein said material is wax and said release means is heat.

23. The method according to claim 18 wherein said material is gelatin and said release means is heat.

24. The method according to claim 18 wherein said material is selected from the class consisting of sucrose and starch, and the release means is an aqueous solution.

25. The method according to claim 18 wherein the material in which said ligand is encapsulated is glyceryl mono-oleate and release of said ligand is affected by exposing the support matrix to a solution of pH > 6.8.

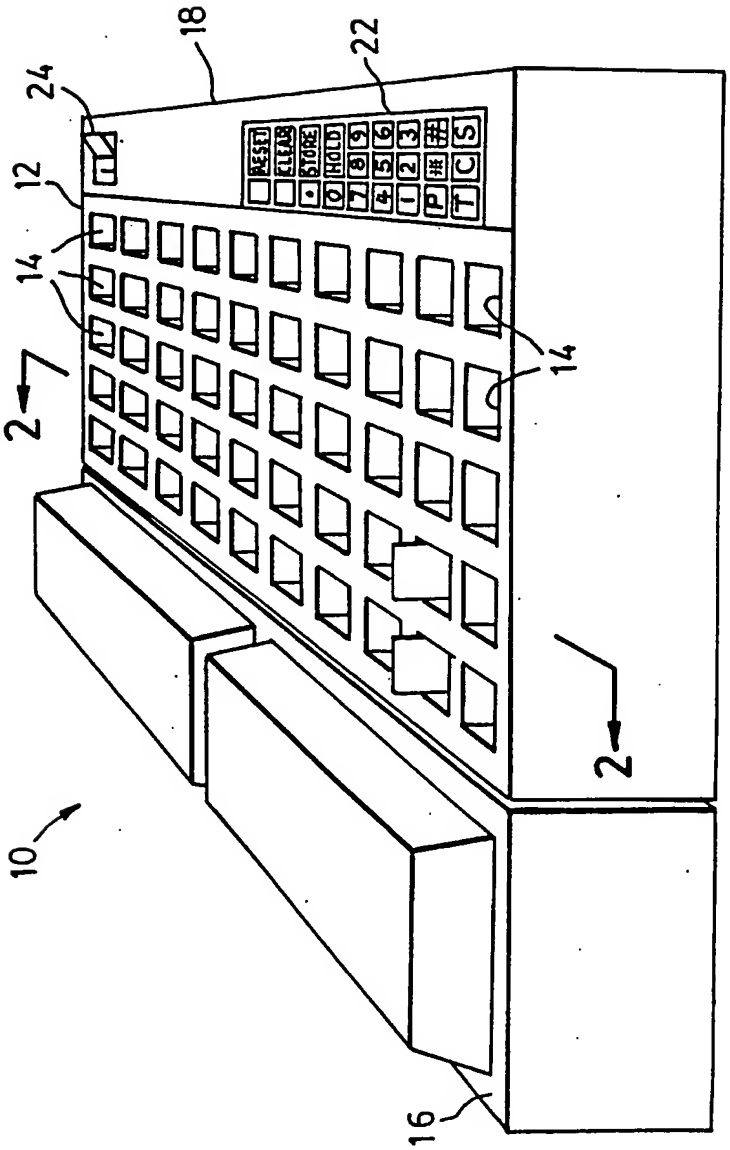
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FIG. 1

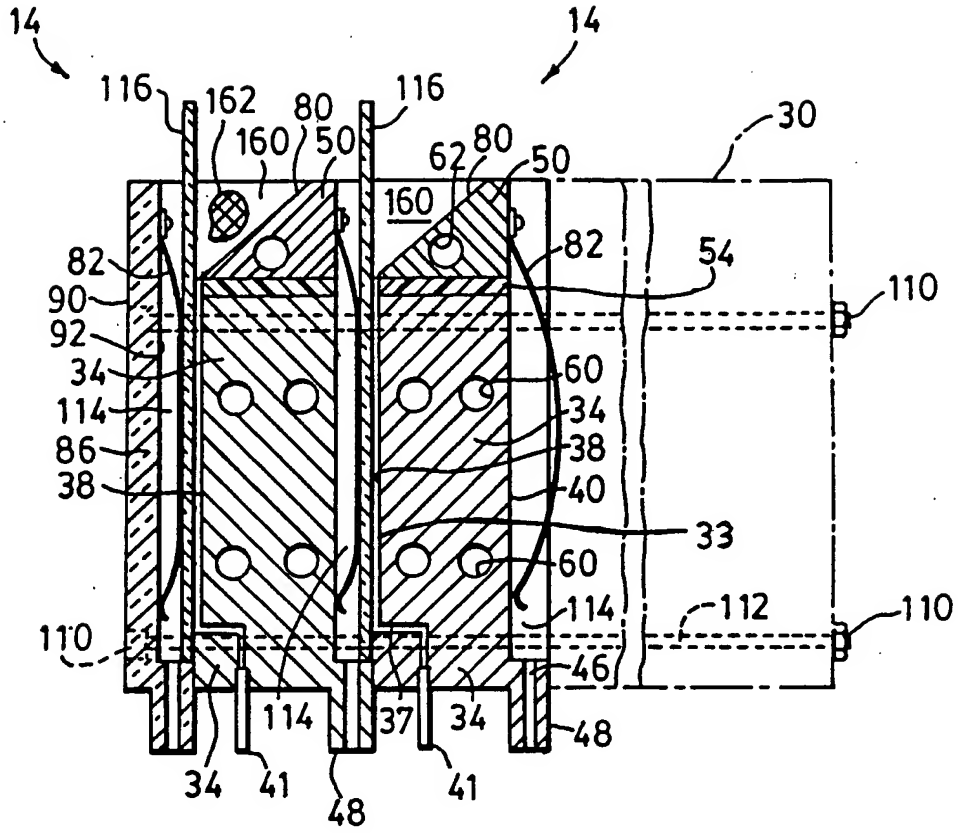
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FIG. 2

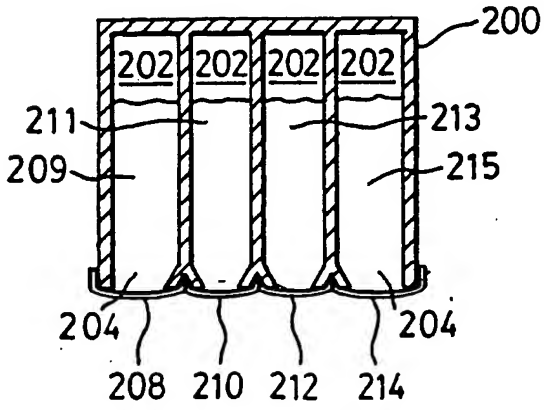
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FIG. 5

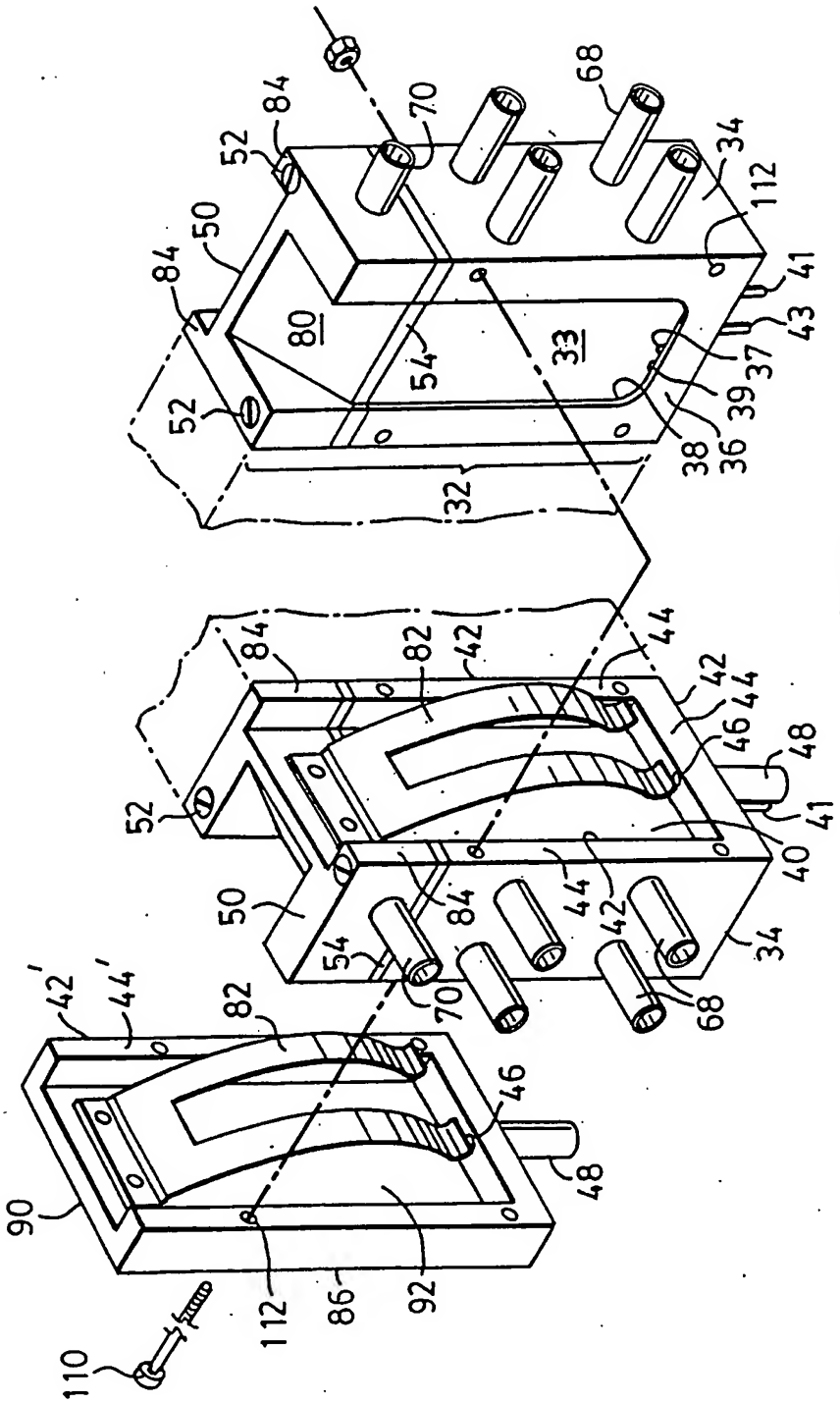
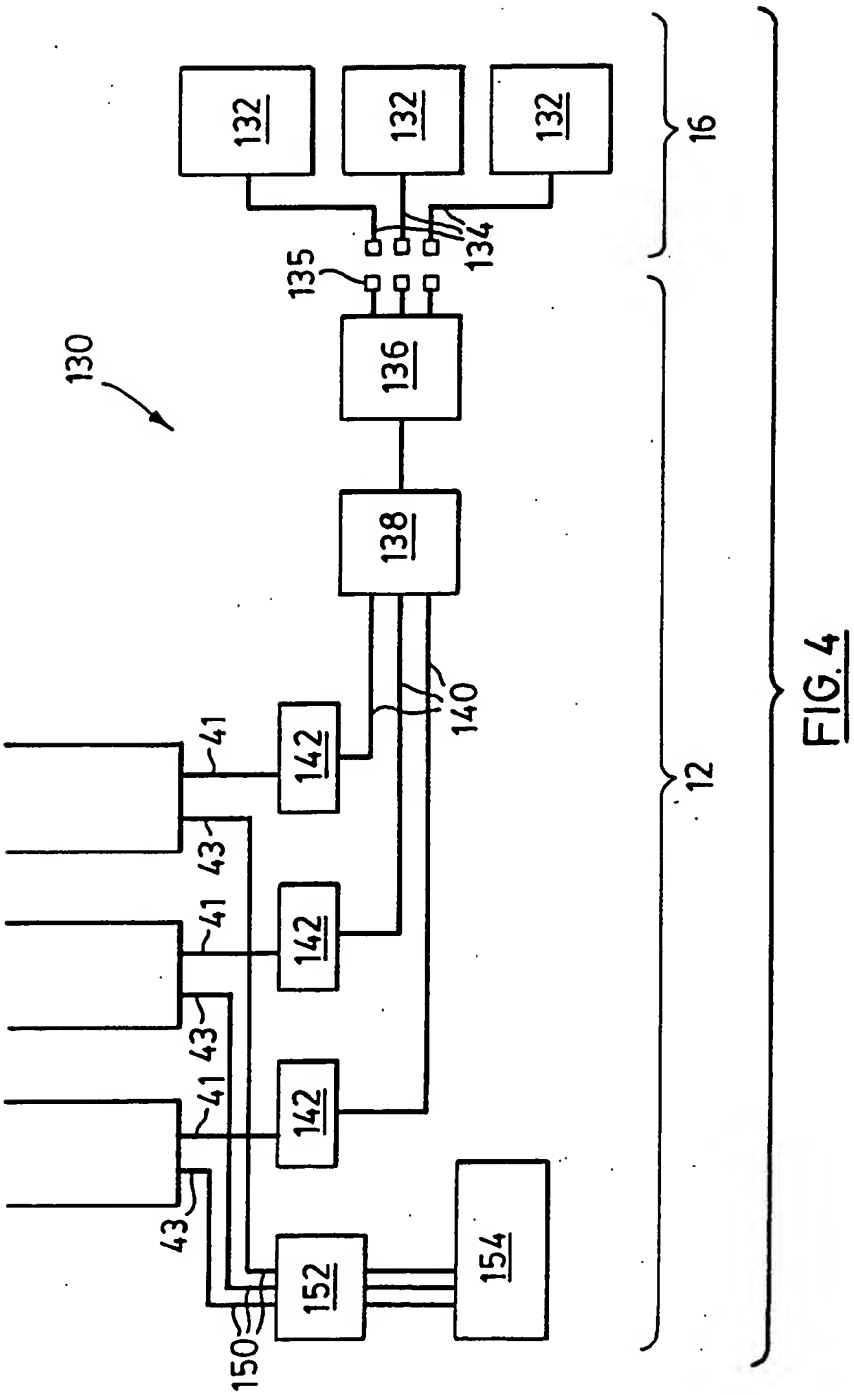

68
70
84
52
34
112
50
84
41
43
80
54
33
37
39
52
38
36
32
84
82
42
44
42
44
46
48
52
41
40
50
84
44
42
54
34
70
68
42'
44'
82
46
48
90
112
86
92
110

FIG. 3


132
132
132
16
134
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130
136
138
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41
142
43
41
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43
41
142
43
152
154
150
12

FIG. 4

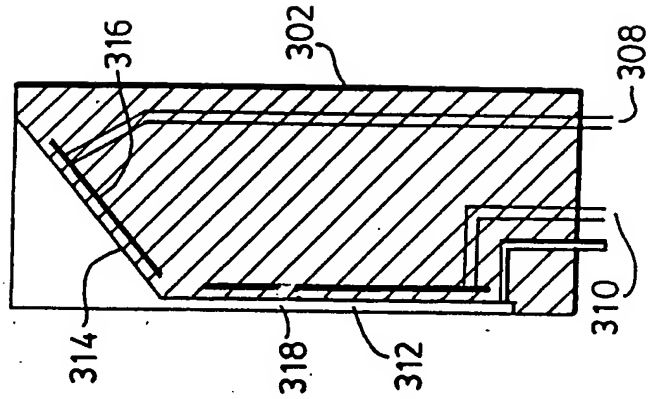

FIG.7

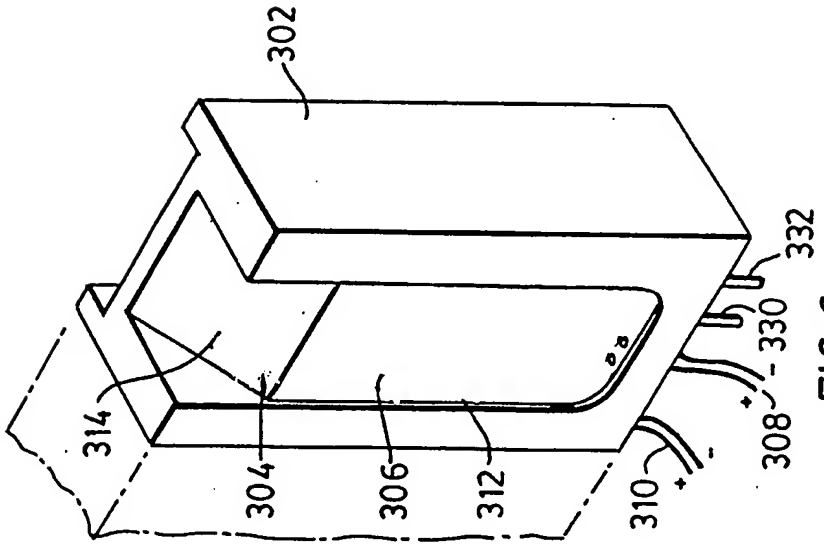

FIG.6